Supplemental Data

A Structural Basis for the Biochemical Behavior of Activation-induced Deoxycytidine Deaminase Class-switch Recombination Defective Hyper-IgM-2 Mutants

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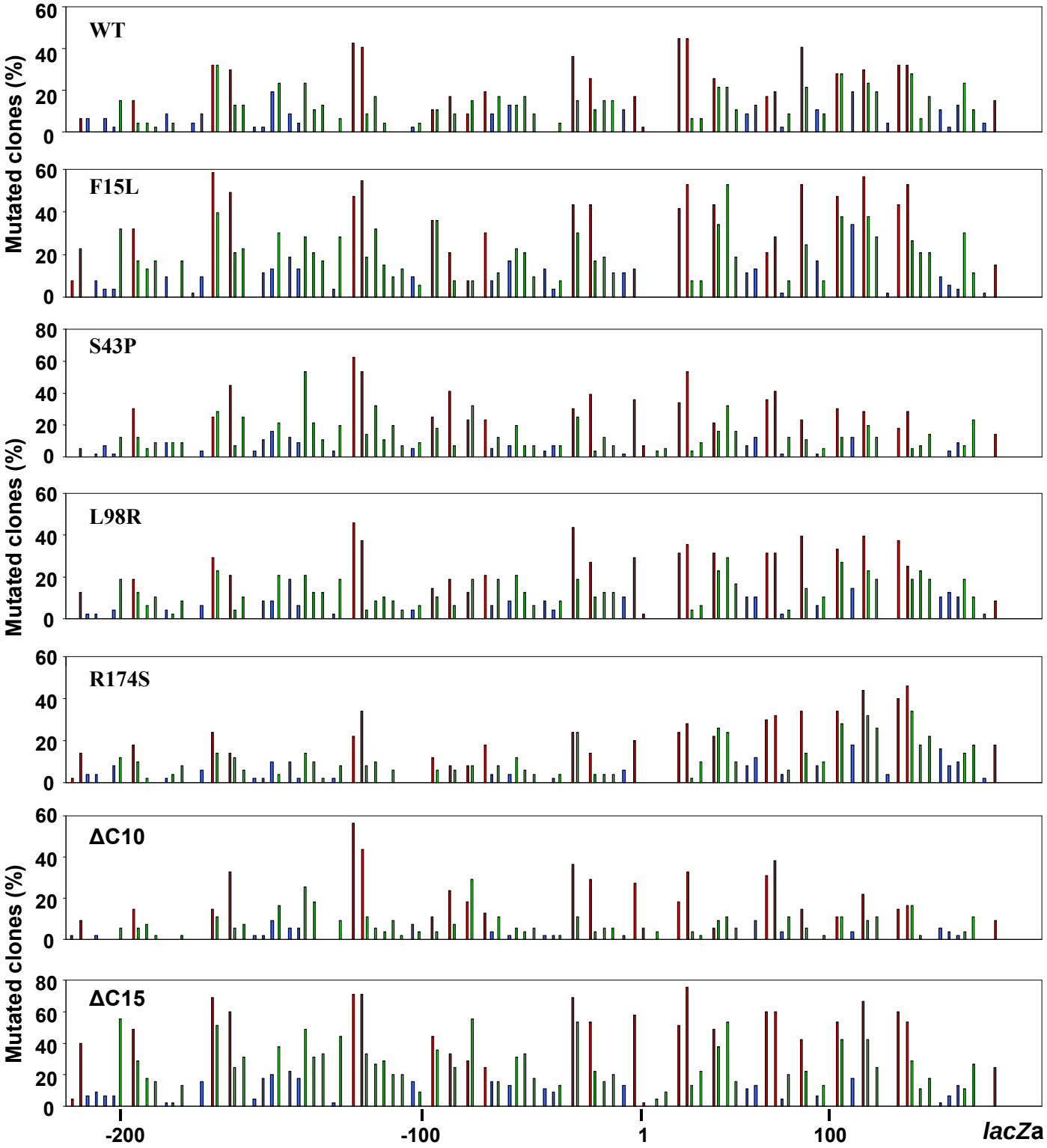
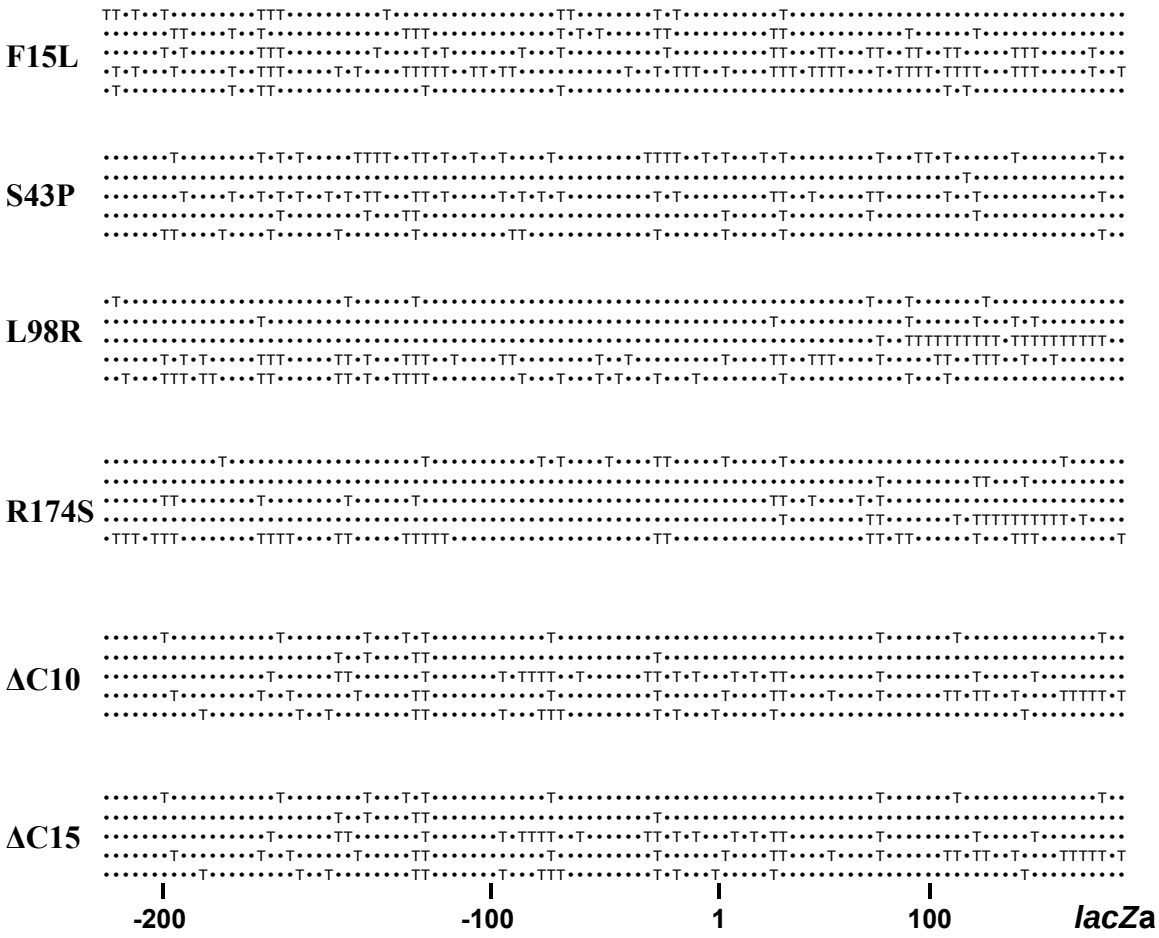
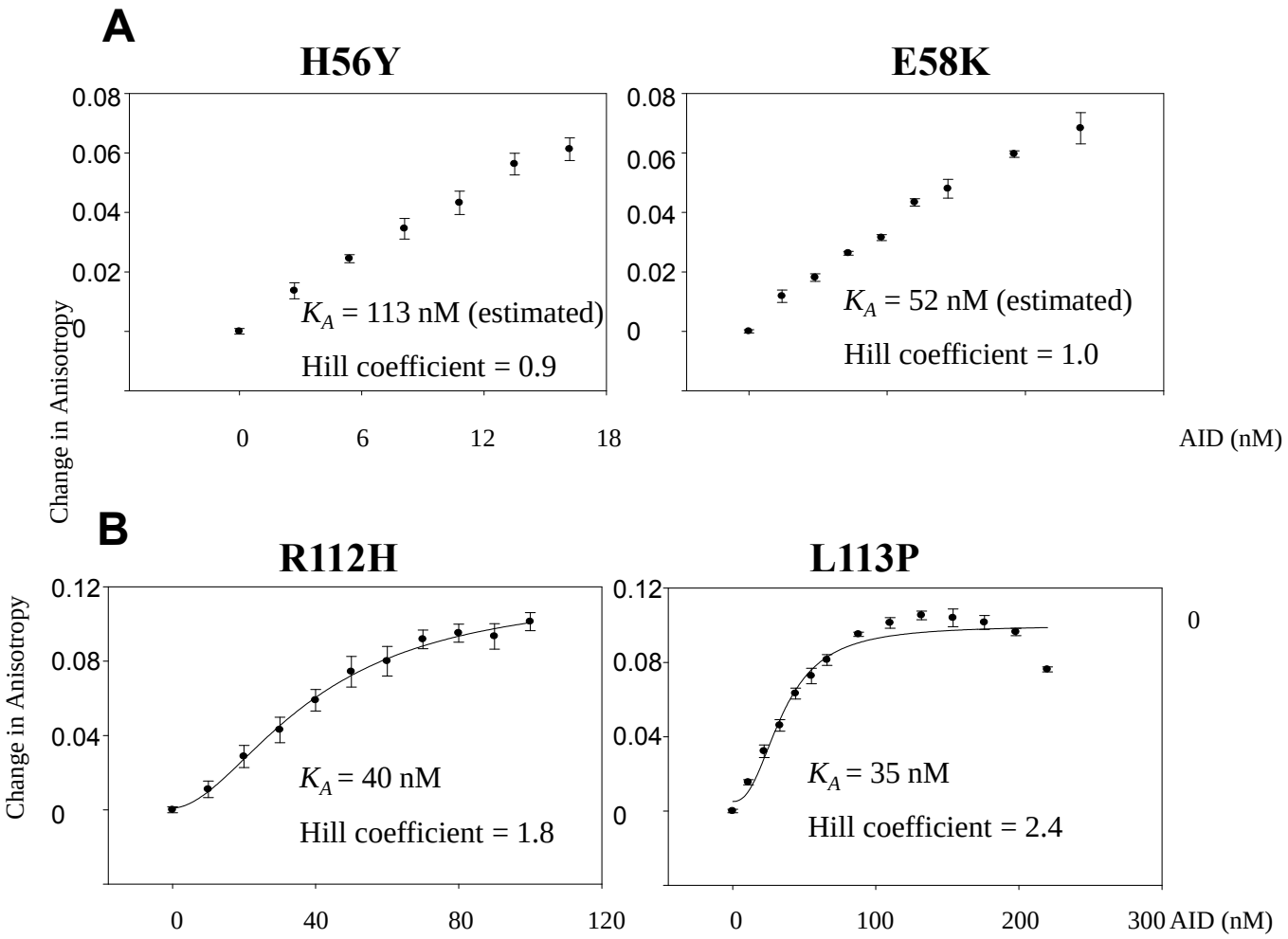


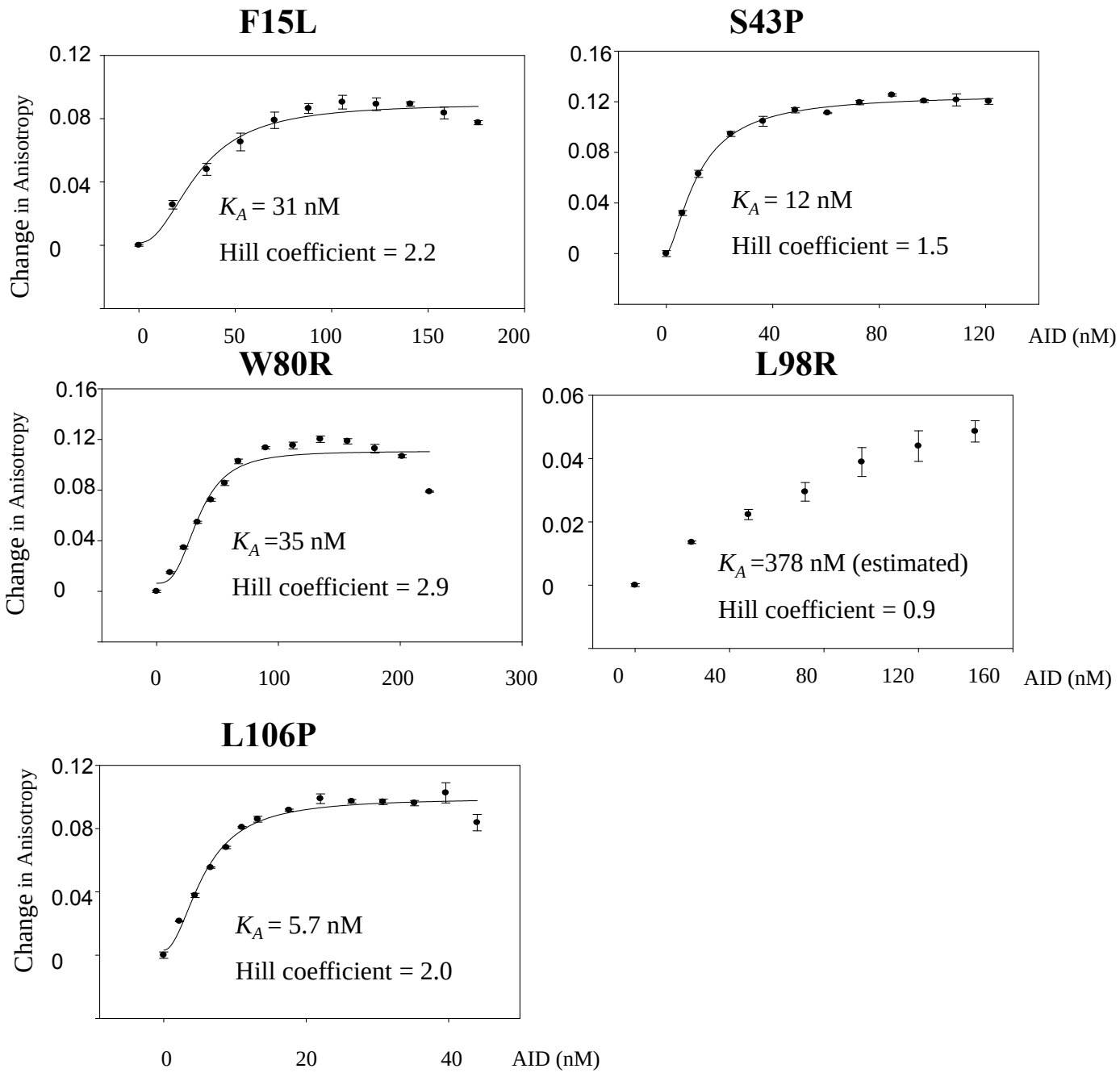
Supplemental Figure S1. *lacZa* C → U deamination spectra of WT and mutant AID at 5 min incubation time. The data for each WT and mutant AID were obtained by sequencing about 50 individual mutant clones. Deaminations resulting in C → T mutations in the *lacZa* target sequence are identified as clear or light blue plaques, whereas non-mutated phage appear as dark blue plaques (see Methods). Each colored bar represents a percentage of mutated phage clones with a C → T mutation at the indicated position on the *lacZ*a target sequence (-217 to +149). *Red* bars identify C deaminations occurring in 5'WRC hot-spot motifs, *blue* bar represent 5'SYC cold-spot motifs, and *green* bars represent neutral motifs (neither WRC nor SYC).



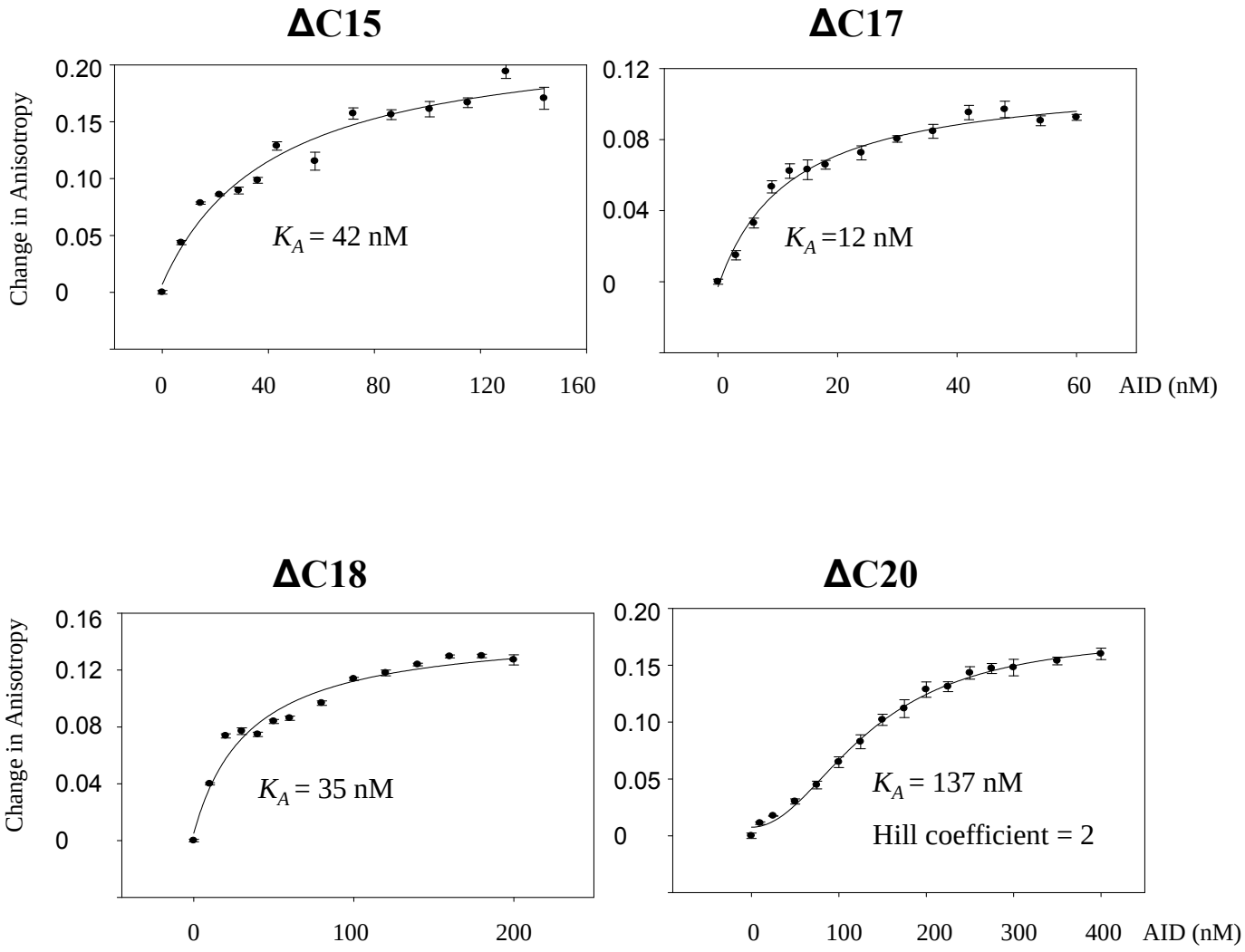
Supplemental Figure S2. Representative mutated M13 phage clones catalyzed by mutant AID on the ssDNA *lacZa* target. Deamination by WT and mutant AID were detected as C → T mutations by sequencing individual phage DNA isolated from the mutant (white or light blue) M13 plaques. For each AID mutant, 5 mutated clones are shown. T represent deaminated C and dots "." denote non-deaminated C on the *lacZa* target. There are106 C target sites on the *lacZ*a.



Supplemental Figure S3. ssDNA binding activity of HIGM-2 mutant AID**. A,** Binding of active site mutants (Class I) H56Y and E58K to a Fluorescein-labeled 36-nt oligonucleotide substrate. Due to low concentration of the mutant proteins, the changes in rotational anisotropy did not did not reach saturation, therefore the K_A were estimated by fitting to hyperbolic binding curve. **B,** Binding of HIGM-2 Class II mutants (R112H and L113P) that affect AID substrate interaction. The changes in rotational anisotropy were plotted with increasing AID concentrations and fit to either a sigmoidal binding curve. Values for each data point represent the mean ± S.E which were determined from 3 independent measurements.



Supplemental Figure S4. Binding of HIGM-2 Class III mutants with mutations important for AID structure. The changes in rotational anisotropy were plotted with increasing AID concentrations and fit to either a sigmoidal binding curve. Values for each data point represent the mean ± S.E which were determined from 3 independent measurements.



Supplemental Figure S5. Binding of C-terminal deletion AID (Class IV). The changes in rotational anisotropy were plotted with increasing AID concentrations and fit to either a rectagular hyperbola (ΔC_{15}, ΔC_{17} and ΔC_{18}) or sigmoidal binding curve (ΔC_{20}). Values for each data point represent the mean ± S.E which were determined from 3 independent measurements.